

## Scanning electron microscopy of human dermal fibrous tissue

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### INTRODUCTION

Microscopic studies of the form and arrangement of fibres in the dermis of human skin have been reported, but there is no unanimity among their conclusions. The discrepancies may be due to lack of experimental control or to limited resolving power of the instruments used. This paper describes the results of a systematic scanning electron microscope (SEM) study of the connective tissue in the dermis of human skin specimens prepared under controlled conditions.

The dermis of human skin is usually described as having two structurally distinctive layers: the papillary layer and the reticular layer (Montagna, 1962). The papillary layer underlies the epidermis and is composed of open networks of fine fibres. Below this the reticular layer, composed of densely intertwining coarse fibres, forms the main body of the dermis. In contrast to this classical description, Craik & McNeil (1965) identified three structural layers: the papillary layer, the mid-zone, and the deep zone. The mid-zone was described as having a more compact arrangement of fibres than the deep zone.

The dermal fibres form a complex microarchitecture although most lie nearly parallel to the surface (Montagna, 1962). The directional arrangement of the reticular layer fibres is described by some as apparently random (Gibson, Kenedi & Craik, 1965), while others report regular lattice patterns (Langer, 1861; Cox, 1941; Ridge & Wright, 1966). Some of these studies were undertaken to explain how elliptical puncture wounds are produced by conically pointed stabbing instruments. Skin sections taken in various planes around the punctures showed rhomboidal arrangements of fibres, and this was assumed to be the natural configuration. However, it is likely that local stretching of the skin, prior to rupture, caused severe distortion of the fibre network (Gibson *et al.* 1965), and the arrangement subsequently seen should not be taken to be natural.

Connective tissue in the dermis is composed of the fibrous proteins collagen, elastin, and reticulin. Collagen is predominant (77 % of fat-free dry weight), elastin accounts for 4 %, and reticulin 0.4 % (Weinstein & Boucek, 1960; Montagna, 1962). These fibres are found throughout the dermis. Little has been said about the form of individual fibres in the papillary layer apart from their small diameters. In the reticular layer the collagen networks are composed of fibres ranging in width from 10 to 40  $\mu\text{m}$  (Gross & Schmitt, 1948). These are composed of still narrower fibrous elements, the fibrils. Montagna (1962) described the individual fibres as branching wavy bands. Branching, he observed, was due to separation of the component fibrils

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into smaller bundles. In contradiction to this Gibson *et al.* (1965) observed dermal collagen fibres to be unbranched filaments. Elastin fibres have no fibrillar sub-structure and are amorphous in nature. In the reticular layer they have been described as branching, cylindrical or ribbon-like in form, with widths ranging from 0.5 to 8  $\mu\text{m}$  (Montagna, 1962). Gibson & Kenedi (1967) noted that they were spirally looped around collagen fibres and formed 'end to side' junctions between each other. Reticular fibres are similar to collagen fibres, of which they may be a precursor (Gross, 1950). Little is known of their form as they constitute only a small part of the skin.

Meaningful studies of the microarchitecture and form of dermal fibres require that these features must not be significantly altered by techniques of specimen preparation. Leather technologists studying fibre formations in animal skins have found that embedding in paraffin wax causes fibre shrinkage and distortion of the microarchitecture (Dempsey, 1968). The impregnation temperature of paraffin wax is about 60 °C, while the thermal shrinkage temperature of collagen is 58 °C. Many of the previous studies of dermal fibres have used paraffin wax techniques (Cox, 1941; Craik & McNeil, 1965; Gibson *et al.* 1965; Ridge & Wright, 1966; Carr, 1970; Dawber & Shuster, 1971).

The scanning electron microscope provides a convenient means of examining microscopic surface topography. The instrument has been used to examine such skin components as cells, hair, hair follicles, glands and blood vessels (Millington & Brown, 1970). SEM studies of dermal fibrous tissue have presented miscellaneous observations (Finlay, 1969; Carr, 1970; Dawber & Shuster, 1971) and high magnification detail (Finlay & Hunter, 1971; Finlay, Hunter & Steven, 1971), but no complete SEM description of the dermal fibres has previously been made. In the study reported here the SEM was used to examine the microarchitecture of fibres through the thickness of the dermis, the directional arrangement of the fibres, and their individual form.

#### MATERIAL AND METHODS

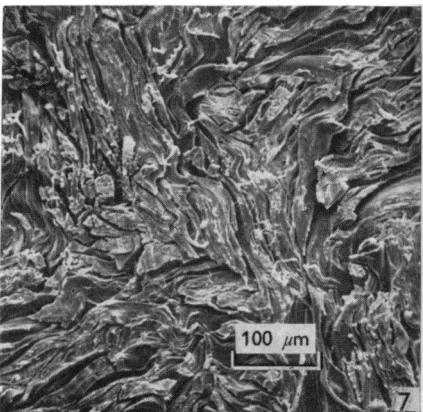
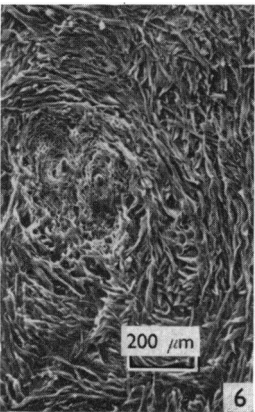
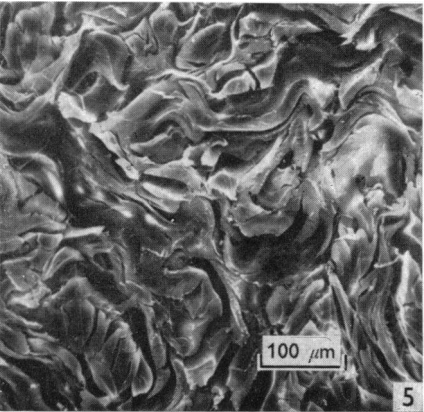
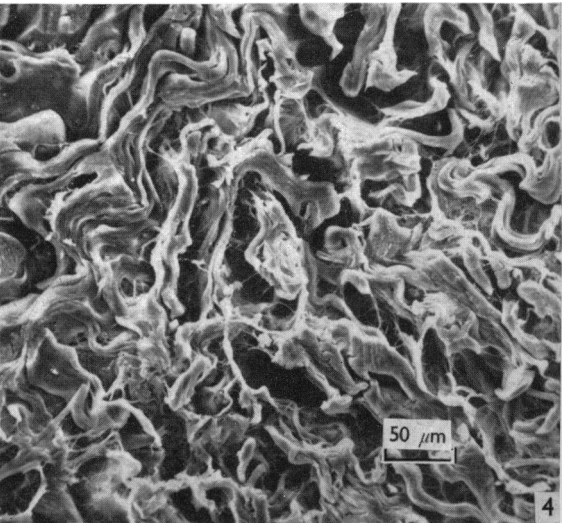
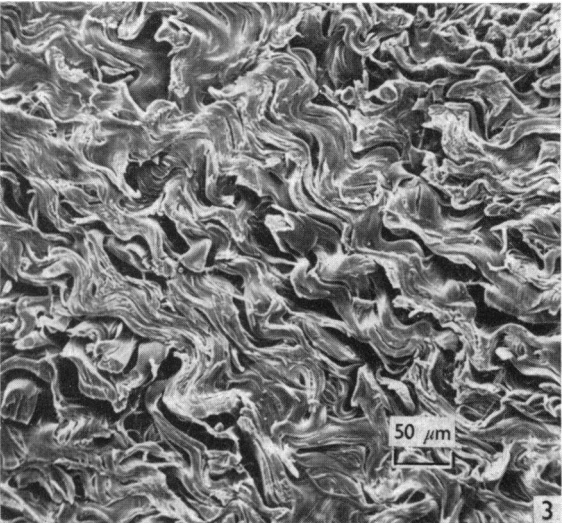
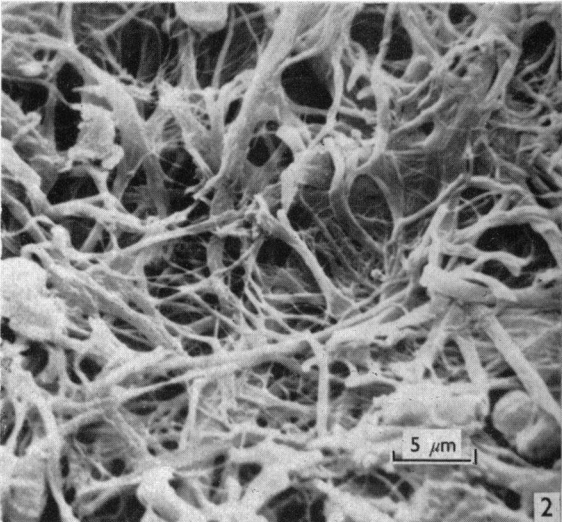
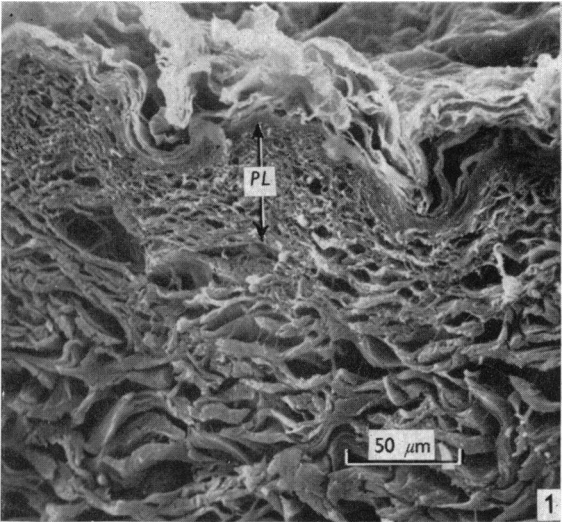
Skin from thirty adults, aged 30–85 years, was obtained prior to autopsy. Samples were excised from the abdomen in the region midway between the xiphisternum and the umbilicus. To prevent stretching the skin during the cutting process, a brass frame (75 mm  $\times$  75 mm) was attached by an adhesive to the skin before excision. The sample was taken by carefully cutting around the frame and deeply undercutting the subcutaneous fat. The orientation of the sample was recorded by markings on the frame. While the sample was still attached to the frame, subcutaneous fat was removed by taking horizontal slices through the fat layer with a Gibson–Ross dermatome until the dermis was exposed. A special cutting board was used in this process to prevent distortion of the sample. The frame was mounted fat layer upwards on a mating wire mesh platform with the epidermal surface resting on the mesh. Suction was applied through the mesh to support the skin during the cutting process. Shearing strain was further minimised by lubricating the sawing action of the blade by sprinkled water. Removal from the frame involved careful manipulation of the scalpel blade between adhesive and frame. The sample was then fixed in 10 % phosphate buffered formol saline for 10 days.

Internal components of the skin were exposed by a freezing microtome technique. Fixed tissue was cut by a scalpel into specimens measuring 12 mm  $\times$  7 mm, and each specimen was mounted on ice contained in a box (25 mm  $\times$  25 mm  $\times$  25 mm) made of moulded wax, which acted as a thermal insulator. It was quickly frozen by pouring Arcton 12 over it until it was rigid and attached to the ice. The wax box was mounted on the holder of a Leitz Sledge Microtome, Type 1300. The rigid condition of the specimen was maintained by sprinkling refrigerant on it during the sectioning process. Planar sections, 20  $\mu$ m in thickness, were cut but these were discarded as it was the exposed surface on the bulk specimen which was to be examined in the SEM. Four SEM specimens were obtained from each sample. Three were cut parallel to the surface exposing the dermis at the following levels: (a) just below the epidermis, (b) the mid-region of the dermis, and (c) deep in the dermis. The fourth specimen was cut perpendicular to the surface to expose a complete edge section.

Sectioned specimens were immersed in an aqueous solution of hyaluronidase (20000 i.u./litre) for 24 hours at 37 °C with occasional agitation. Dehydration in alcohols followed in the sequence of concentrations 30, 50, 70, 90 %, each for 30 minutes, and finally in 100 % for 24 hours. Alcohol was removed by placing specimens in a vacuum desiccator for 48 hours.

After attachment to the SEM specimen stub the specimens were coated with a 20 nm layer of gold palladium in an Edwards vacuum coater with an offset rotating specimen holder (for even coating of the rough surface). The conducting path from the coating to the stub was completed by use of a conducting paint. Specimens were examined in a Cambridge Stereoscan Mark 2A scanning electron microscope. Micrographs were recorded on 120 size Ilford FP4 film using a Zenza Bronica camera.

When the mid-dermal region was examined at  $\times 200$  magnification, the fibres within the field of view frequently appeared oriented although the direction of orientation varied from site to site on the specimen. A survey was made to determine if a preferred orientation existed across the specimen. During the survey specimens were inclined perpendicular to the electron beam to avoid the foreshortening of the true shape of the specimen which occurs when the normal specimen tilt angle of 45° is used. Magnification was kept constant on each specimen at  $\times 200$  approximately. The direction of the cranio-caudal meridian of the donor was positioned vertically on the SEM display screen. At each site a visual assessment was made of the fibre orientation within the field of view. To allow quantitative analysis of fibre orientation eleven symbols were chosen to represent the range of fibre patterns which could occur. The symbols are shown in Fig. 8. The direction of the lines composing the symbols indicate the directions of fibres observed within the field of view at a randomly chosen site. Examples of three fibre arrangements and the symbols ascribed to them are given in Figs. 3, 5 and 7. On each specimen fifty sites were chosen by random manipulation of the specimen stage controls of the SEM, so that all areas of the specimen surface were represented in the survey. The operator only intervened in the random selection of sites to avoid non-fibrous structures such as fat cells, glands, etc. Specimens from seven individuals were included in the survey.



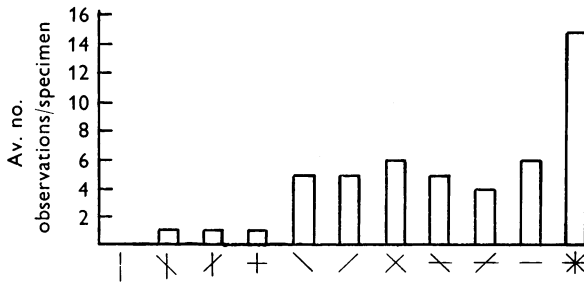


Fig. 8. Histogram showing the average distribution of observations of fibre pattern symbols per specimen obtained from analysis of seven abdominal skin specimens sectioned parallel to the surface through the mid dermis (fifty observations per specimen). The lines of the symbols simulate the major fibre orientations within the 0.5 mm square microscopic field.

### RESULTS

Three structural zones are distinguished in the fibrous tissue through the thickness of the dermis. Each zone is characterized by fibre form and arrangement. Immediately underlying the epidermis and following its undulating form, is the layer of fine fibres called the papillary layer (Fig. 1). These fibres are typically 0.3 to 3  $\mu\text{m}$  in width, and form a relatively open network in which no regular arrangement is apparent (Fig. 2). The deeper part of the dermis, forming the bulk of abdominal skin and usually termed the reticular layer, is composed of coarse fibres 10–40  $\mu\text{m}$  in width. These are more compactly arranged in the superficial two-thirds, referred to now as the mid zone of the dermis (Fig. 3), than in the deeper aspects where a looser arrangement prevails, called the deep zone (Fig. 4). Aggregations of fat cells are found in greater abundance within the networks of the deep zone.

At low magnifications the fibres of the mid zone and deep zone appear to be arranged in a haphazard multidirectional system (Fig. 5). The only exception to this non-regularity is the concentric arrangement which often surrounds hair follicles (Fig. 6). In specimens sectioned through the mid zone parallel to the surface the fibres within the microscopic field (0.5 mm square) at approximately  $\times 200$  magnification frequently appear to be partially oriented (Figs. 3, 7), but the direction of orientation varies between sites. The results of the survey of fibre orientation are summarised in histogram form in Fig. 8, which shows the average distribution of

Fig. 1. An edge section of abdominal skin showing, from the top, the epidermis, the papillary layer (PL), and coarse fibres of the mid zone.  $\times 300$ .

Fig. 2. Fine fibres of the papillary layer form a relatively open network.  $\times 2200$ .

Fig. 3. Section parallel to the surface through the mid zone of the dermis showing the densely packed coarse fibres. Fibre arrangement denoted by: \.  $\times 175$ .

Fig. 4. Section parallel to the surface through the deep zone of the dermis showing the loose arrangement of coarse fibres.  $\times 185$ .

Fig. 5. Section parallel to the surface through the mid zone showing a multidirectional fibre system with no preferential orientation. Fibre arrangement denoted by: \*.  $\times 120$ .

Fig. 6. Concentric arrangement of fibres around hair follicles.  $\times 47$ .

Fig. 7. Partial orientation of fibres in the mid zone. Fibre arrangement denoted by: X.  $\times 120$

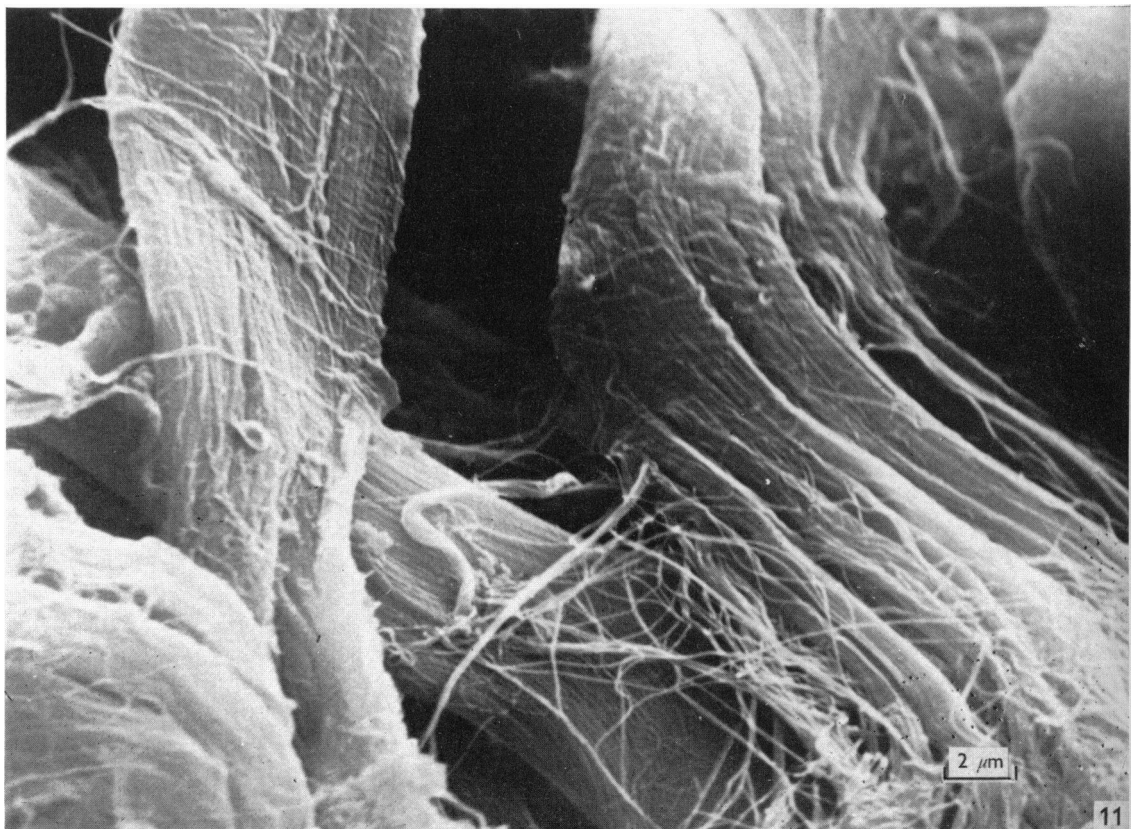
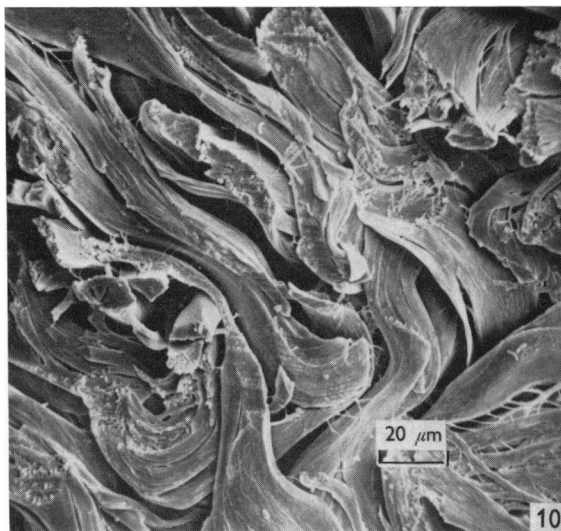
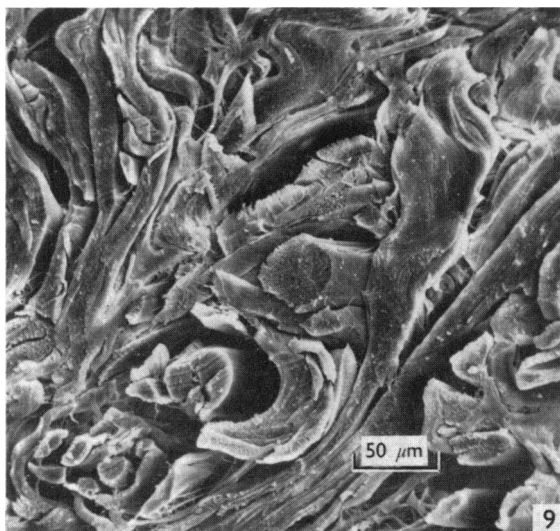


Fig. 9. Fibres in the mid zone vary in breadth and cross-section.  $\times 240$ .

Fig. 10. Fibres in the mid zone have various forms and intertwine in a dense irregular network.  $\times 505$ .

Fig. 11. Most fibrils lie in parallel arrays along the fibre, others lie around the fibre and between fibres.  $\times 4800$ .

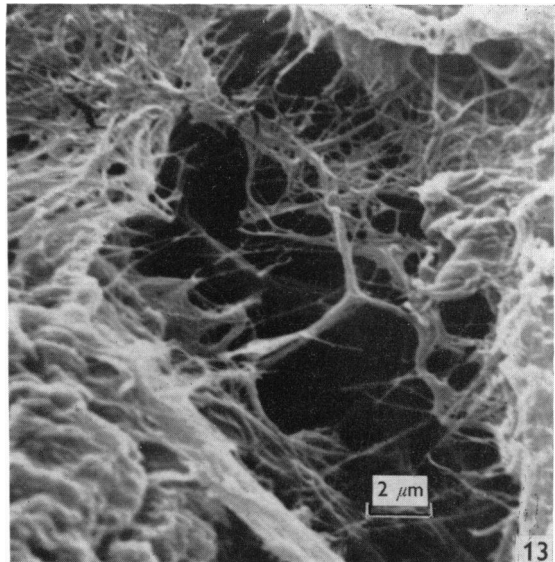
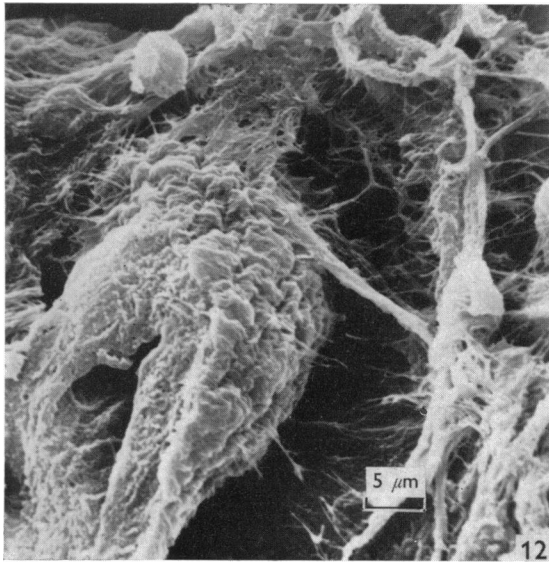


Fig. 12. A blood vessel, probably an arteriole, connected by fine fibres to the surrounding coarse fibre network.  $\times 1560$ .

Fig. 13. A detailed view of the fine fibres around the blood vessel shown in Fig. 12. 'End to side' junctions are common.  $\times 4150$ .

fibre patterns per specimen. The 'no preferred orientation' pattern is observed more frequently than any other individual pattern. Amongst the other pattern forms more show predominant orientation close to the transverse axis of the abdomen than show predominant cranio-caudal orientation; that is, there is preferential orientation of fibres across the abdomen although the network is multidirectional and irregular in structure.

In the mid and deep zones aggregations of fibres take various forms, ranging from flat ribbon-like bundles to those with almost circular cross-sections (Figs. 9, 10). The breadth and shape of a bundle change along its length because of branching and interconnexions between fibres. The only age-related change in fibre form within the age range studied appears to be that the fibres become progressively straighter with increasing age.

At higher magnifications the fibrillar composition of many of the fibres is discernible. Most fibrils are aligned in parallel arrays along the length of the fibre (Fig. 11). A few lie in a haphazard arrangement on the fibre surface. The spaces between adjacent fibres are bridged at intervals by an open network of fibrils. Hair follicles, glands and blood vessels are surrounded by a system of fine fibres. These appear to form attachments to the coarse fibre network comprising the bulk of the dermis (Figs. 12, 13).

## DISCUSSION

In a preliminary study (Finlay, 1969) it was assumed that hyaluronidase treatment was necessary to remove ground substance from the interstices of fibre networks. Satisfactory results were obtained even with fixed material, although the mode of action was inexplicable. More recent studies have shown that amorphous debris is indeed removed by the treatment and that this is probably a mechanical washing action rather than an enzymic one (Brown, 1971).

The classical description of the dermis identifies two fibre layers: the papillary layer and the reticular layer. In this study the reticular layer is further subdivided into the mid zone and deep zone of the dermis. These regions are characterized by the density of the fibre network, which is greater in the mid zone. This classification is in agreement with that of Craik & McNeil (1965).

Fibres in the mid zone were observed to form a multidirectional system in which there was no regular arrangement. A previous study of unpunctured skin also reported an apparently random distribution of fibres (Gibson *et al.* 1965). However, when fibre arrangements were quantitatively assessed in this study, a preferential orientation in the horizontal plane was found. Previous studies, which described regular lattice arrangements of fibres, related the long axis of rhomboidal mesh to the direction of puncture slits. Since the regular lattice arrangement of fibres in the dermis of abdominal skin seems to be observed only after puncturing with a conically pointed instrument, as indeed Cox (1941) has stated, it is likely that the natural fibre configuration in relaxed skin is disrupted by the puncturing process.

Mechanical studies of abdominal skin have shown that skin is more extensible in the cranio-caudal direction than in the transverse direction (Daly, 1966). It was suggested that the anisotropy was due to preferential fibre orientation in the direction of minimal extensibility. The findings of the present study support this hypothesis. When skin is progressively stretched the dermal fibres re-orientate, straighten and become aligned in the load direction, after which further extension is limited by the inextensibility of the collagen fibres (Craik & McNeil, 1965). Since the fibres are partially oriented across the abdomen in the unstressed condition the extension 'limit' will occur at the lowest strain level when the load axis is across the abdomen and greatest when it is in the cranio-caudal direction.

During life the response of the skin to mechanical stress is complicated by its stretched condition in which the resting tension is anisotropic (Gibson & Kenedi, 1967). When planning an incision surgeons attempt (other factors permitting) to cut along the direction of maximum tension, as this results in minimal tension across the healing wound. As no universal pattern of maximum tensions exists (Gibson & Kenedi, 1967) wrinkle lines are advocated as the lines of choice, as wound scars are hidden by the wrinkles, and these may also indicate the direction of maximum tension, as Cox (1941) has suggested. If an area of skin is to be excised then the extensibility of the surrounding skin, which is stretched to make good the defect, must also be taken into consideration. In this respect the skin is more extensible in the cranio-caudal direction.

Identification of the fibre components of the dermis (collagen, elastin and reticulin) were not made in this study, which was restricted to fibre arrangement and form.

However, it is likely that fibres composed of fibrils were collagenous. When operating the SEM in the secondary electron emissive mode for topographical studies, it is not possible to label components, as can be done in optical microscopy by selective staining. It may be possible to selectively stain tissue components by electron-excited luminescent dyes and to identify them in the SEM using the cathodoluminescent mode. More basic research needs to be done in this area.

#### SUMMARY

Fibrous tissue in the dermis of human skin was examined by scanning electron microscopy. Specimens of abdominal skin were obtained post mortem and were prepared for microscopy, great care being taken to minimize distortion. Observations are reported on the variation in the fibre network structure through the thickness of the dermis, the directional arrangement of the fibres in the mid-dermis, and the form of individual fibres.

Three structural zones were identified: the papillary layer – a thin layer of fine fibres adjacent to the epidermis; the mid zone – a thick layer of densely packed coarse fibres; and the deep zone – a loosely arranged layer of coarse fibres.

The bulk of the dermis was composed of a multidirectional system of fibres with no regular arrangement. Quantitative analysis of fibre orientation showed preferential orientation across the abdomen. This is discussed in relation to previous structural and mechanical studies.

Individual fibres were found to be branching. Fibrils were observed to lie along the fibre, around the surface and between fibres.

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